## **Biostatistics**

Statistical computing Luca Alberto Rizzo









## Agenda

- 1 Introduction to R
- 2 Penguins dataset
- 3 Histogram, barchart & boxplots
- 4 Statistical tests for means and proportions
- **5** ANOVA
- 6 Conclusions



# 1 Introduction to R

## Introduction to R: why learn R?



Lingua franca for statistical computing

why learn

Free and open source



Latest model/tech implemented

Used in industry & academia

Robust

visualization

libraries

Large and active community

#### Introduction to R: what is R?

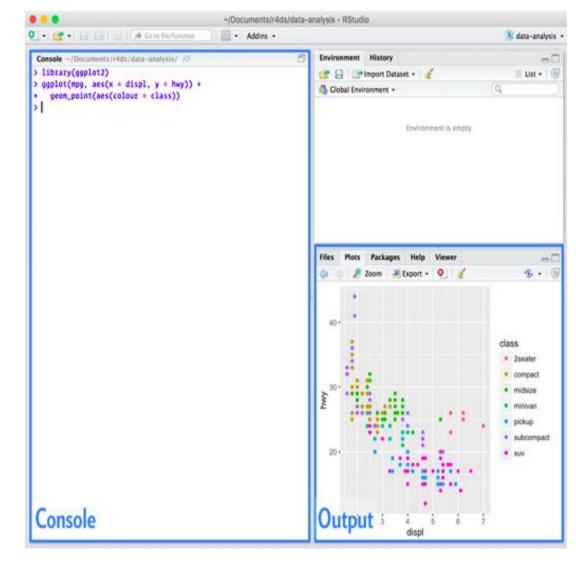


#### Introduction

- R is a language and environment for statistical computing and graphics
- R is developed and maintained by the R foundation
- R has >17,000 packages, with additional functions

#### Installation

- R can be installed easily via this link
- RStudio is an integrated development environment, or IDE, for R programming: It can be found <u>here</u>



## Introduction to R: "hello world" and tidyverse



- 1. Let's start by greeting the whole world!
- 2. Install <u>tidyverse</u>, a <u>bundle of ~25 packages</u> designed to help with data management, reproducibility and multi-level programming
- 3. Let's compute our first mathematical operation in R (mean)

4. Assign a vector and compute its mean with the built-in R function

```
> print("hello world")
[1] "hello world"
```

> install.packages("tidyverse")
Installing package into '/home/lumaca/R/x86\_64-pc-linux-gnu-library/4.2'
(as 'lib' is unspecified)

Take away:
Do not reinvent the wheel!

## Introduction to R: data types

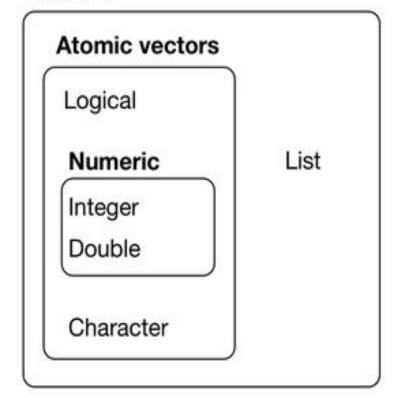


NULL

#### Vectors and types in R

- 6 atomic (simple) vectors: logical, integer, double, character, complex, raw
- Integers and doubles are numeric
- c operator builds non-atomic vectors
- a list is a heterogenous (recursive) vector

#### Vectors



Source: Figure 20.1 "R for Data Science"

## Introduction to R: operators



#### Operators in R

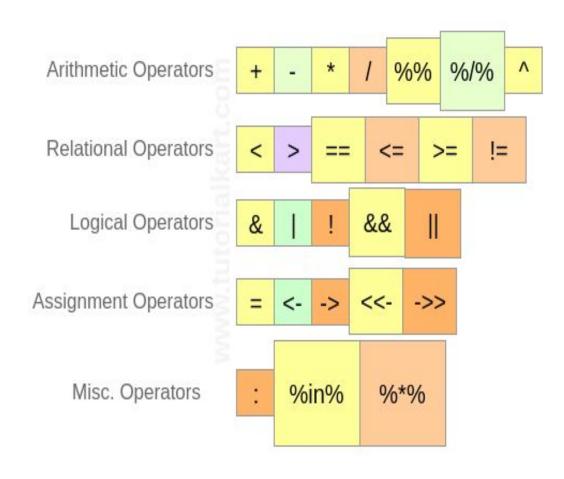
• the assignment operator in R is both:

== checks equality between 2 elements

Assign with <-

Assign with =

Checking equality with ==



Souce: tutorial kart website

#### Introduction to R: dataframe & tibbles



A dataframe is a 2D data structure in R, a special case of a list which has each component of equal length.

```
> print(penguins)
# A tibble: 344 × 8
                     bill_length_mm bill_depth_mm flipper_length_mm body_mass_g sex
   species island
                                                                                           vear
   <fct> <fct>
                               <dbl>
                                              <dbl>
                                                                <int>
                                                                             <int> <fct>
                                                                                          <int>
                                                                              3750 male
 1 Adelie
                                39.1
                                              18.7
                                                                  181
                                                                                           2007
           Torgersen
 2 Adelie
                                                                              3800 female
           Torgersen
                                39.5
                                              17.4
                                                                  186
                                                                                           2007
                                                                              3250 female
 3 Adelie
           Torgersen
                                40.3
                                              18
                                                                  195
                                                                                           2007
 4 Adelie
           Torgersen
                                NA
                                              NA
                                                                   NA
                                                                                NA NA
                                                                                           2007
 5 Adelie
                                36.7
                                                                              3450 female
           Torgersen
                                              19.3
                                                                  193
                                                                                           2007
 6 Adelie
           Torgersen
                                39.3
                                              20.6
                                                                  190
                                                                              3650 male
                                                                                           2007
 7 Adelie
           Torgersen
                                38.9
                                              17.8
                                                                  181
                                                                              3625 female
                                                                                           2007
 8 Adelie
                                39.2
                                              19.6
                                                                  195
                                                                              4675 male
                                                                                           2007
           Torgersen
 9 Adelie
                                                                              3475 NA
           Torgersen
                                34.1
                                              18.1
                                                                  193
                                                                                           2007
10 Adelie
           Torgersen
                                42
                                              20.2
                                                                              4250 NA
                                                                  190
                                                                                           2007
# ... with 334 more rows
```

This is a tibble, a special version of a dataframe implemented in the tidyverse library

#### Introduction to R: dataframe & tibbles



#### **Summary** prints useful information

#### > summary(penguins) species island bill length mm bill depth mm flipper length mm Adelie :152 Biscoe :168 Min. :32.10 Min. :13.10 Min. :172.0 :124 1st Ou.:39.23 1st Ou.:15.60 1st Ou.:190.0 Chinstrap: 68 Dream Median :44.45 Median :17.30 Median :197.0 Gentoo :124 Torgersen: 52 Mean :43.92 Mean :17.15 Mean :200.9 3rd Qu.:48.50 3rd Qu.:18.70 3rd Qu.:213.0 :59.60 Max. :21.50 Max. :231.0 Max. NA's :2 NA's :2 NA's :2 body mass g sex year Min. :2700 female:165 Min. :2007 1st Qu.:3550 male :168 1st Qu.: 2007 Median :4050 NA's : 11 Median :2008 Mean :4202 Mean :2008 3rd Qu.:4750 3rd Ou.:2009 :6300 :2009 Max. Max. NA's :2

#### Mutate adds columns

#### > mutate(penguins,

+ bill\_ratio = bill\_length\_mm / bill\_depth\_mm)

```
# A tibble: 344 × 9
                    bill_length_mm bill_depth_mm flipper_length_mm body_mass_g sex
   species island
                                                                                        year bill ratio
   <fct> <fct>
                              <dbl>
                                            <dbl>
                                                              <int>
                                                                          <int> <fct> <int>
                                                                                                   <dbl>
 1 Adelie Torgersen
                              39.1
                                             18.7
                                                                181
                                                                           3750 male
                                                                                        2007
                                                                                                   2.09
2 Adelie Torgersen
                              39.5
                                             17.4
                                                                186
                                                                           3800 female 2007
                                                                                                   2.27
3 Adelie Torgersen
                               40.3
                                             18
                                                                195
                                                                           <u>3</u>250 female <u>2</u>007
                                                                                                   2.24
4 Adelie Torgersen
                                             NA
                                                                                        2007
5 Adelie Torgersen
                              36.7
                                             19.3
                                                                193
                                                                           3450 female 2007
                                                                                                   1.90
6 Adelie Torgersen
                               39.3
                                             20.6
                                                                190
                                                                           3650 male
                                                                                        2007
                                                                                                   1.91
 7 Adelie Torgersen
                              38.9
                                             17.8
                                                                181
                                                                           3625 female 2007
                                                                                                   2.19
8 Adelie Torgersen
                               39.2
                                             19.6
                                                                195
                                                                           4675 male
                                                                                        2007
                                                                                                   2
9 Adelie Torgersen
                               34.1
                                             18.1
                                                                193
                                                                           3475 NA
                                                                                        2007
                                                                                                   1.88
10 Adelie Torgersen
                                             20.2
                                                                190
                                                                           4250 NA
                                                                                        2007
                                                                                                   2.08
# ... with 334 more rows
```

#### Filter and select

```
> penguins %>% select(species, island, body_mass_g) %>% filter(species == "Adelie")
# A tibble: 152 x 3
  species island
                    body mass g
  <fct> <fct>
                           <int>
1 Adelie Torgersen
                            3750
2 Adelie Torgersen
                            3800
3 Adelie Torgersen
                            3250
4 Adelie Torgersen
                             NA
 5 Adelie Torgersen
                            3450
6 Adelie Torgersen
                            3650
 7 Adelie Torgersen
                            3625
8 Adelie Torgersen
                            4675
9 Adelie Torgersen
                            3475
10 Adelie Torgersen
                            4250
# ... with 142 more rows
```

#### Group by computes quantities per categorical variable



# 2 Penguins dataset

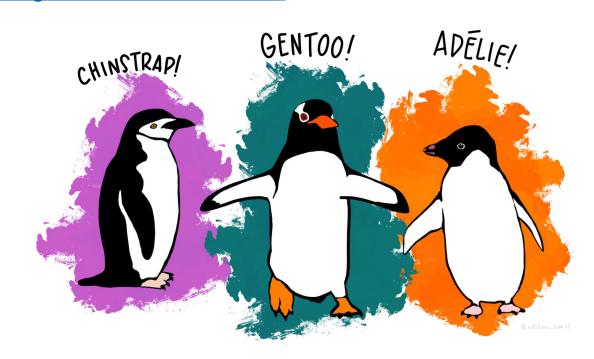
## Penguins dataset: introduction



<u>Penguins dataset</u> was collected by <u>Dr. Kristen Gorman</u> at the <u>Palmer Station, Antarctica LTER</u>, a member of the <u>Long Term Ecological Research Network</u>.

#### > summary(penguins)

```
species
                    island
                              bill length mm bill depth mm
                                                            flipper length mm
                                             Min. :13.10
                              Min. :32.10
                                                                 :172.0
Adelie :152
               Biscoe
                       :168
                                                            Min.
Chinstrap: 68
                       :124
                              1st Qu.:39.23
                                             1st Qu.:15.60
                                                            1st Qu.:190.0
               Dream
        :124
               Torgersen: 52
                              Median :44.45
                                             Median :17.30
                                                            Median :197.0
Gentoo
                              Mean :43.92
                                             Mean :17.15
                                                            Mean
                                                                  :200.9
                                             3rd Qu.:18.70
                                                            3rd Qu.:213.0
                              3rd Qu.:48.50
                                   :59.60
                                                    :21.50
                                                            Max.
                                                                   :231.0
                                             Max.
                              NA's :2
                                             NA's
                                                    :2
                                                            NA's
                                                                   :2
 body mass g
                               vear
                  sex
      :2700
              female:165
                          Min. :2007
1st Qu.:3550
              male :168
                          1st Qu.:2007
Median :4050
              NA's : 11
                          Median:2008
      :4202
                                 :2008
                          Mean
                          3rd Qu.:2009
3rd Qu.:4750
                                 :2009
      :6300
Max.
                          Max.
NA's
      :2
```



Artwork by @allison\_hors

- 334 rows and 8 columns
- 3 species of penguins (Chinstrap, Gentoo, Adelie)
- 3 different islands (Biscoe, Dream, Torgersen)
- 3 factors (species, islands, sex), 2 doubles (bill\_lenght\_mm, bill\_depht\_mm) and 3 integers (flipper\_length\_mm, body\_mass\_g, year)



# 3 Histogram, barcharts, boxplots

## Histogram, barcharts, boxplots: ggplot

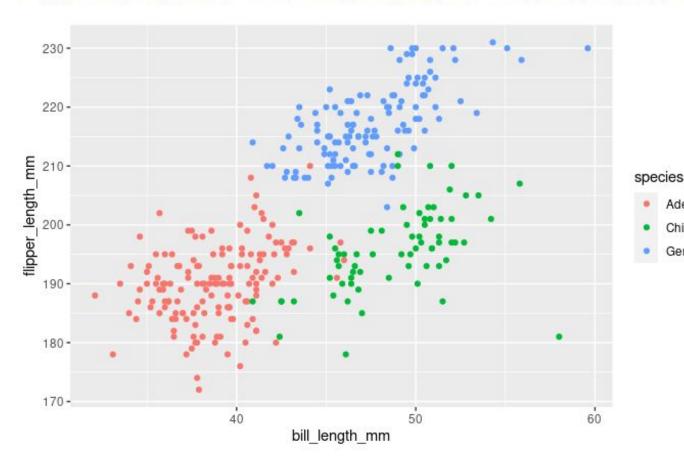


"ggplot2 is a system for declaratively creating graphics, based on The Grammar of Graphics."



> ggplot(penguins, aes(bill\_length\_mm, flipper\_length\_mm, colour = species )) + geom\_point()

Gentoo



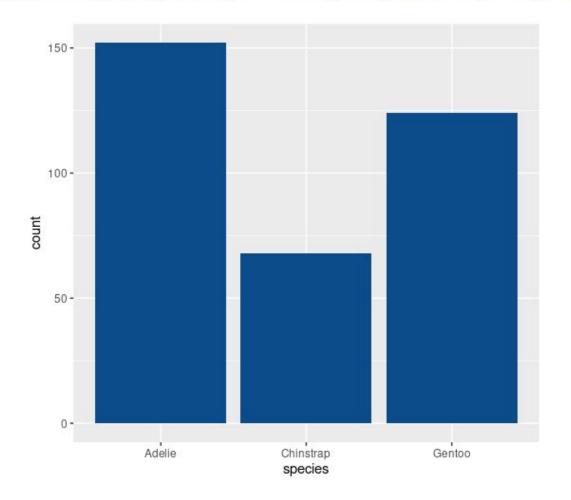
- <u>ggplot(data = NULL, mapping = aes(),</u>
   <u>....)</u> initializes a ggplot object
- <u>aes(x,y,...)</u> "Aesthetic mappings" describes how variables in the data are mapped to visual properties
- layers, like <u>geom\_point()</u>, specify which kind of plot you want to produce
- <u>ggplot cheasheet</u> for more functionalities

## Histogram, barcharts, boxplots: histogram



How many penguins of each species are there in the dataset?

> ggplot(penguins) + aes(x=species) + geom\_bar(stat= "count", fill = "#0c4c8a")



- <u>geom\_bar(mapping = NULL, data = NULL, stat = "count", ...)</u> is the layer which prints "bar charts"
- if geom\_bar(if stat= "count",...) a frequency histogram is plotted

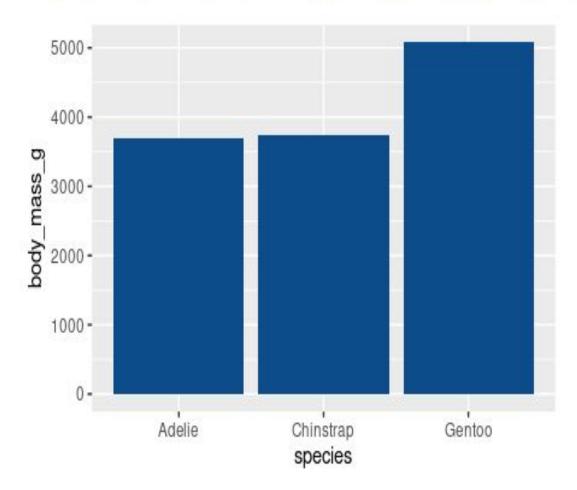
if geom\_bar(aes(y = (..count..)/sum(..count..)), ...), a relative frequency histogram is plotted

## Histogram, barcharts, boxplots: boxplots



What is the average mass of penguins for each specie?

> ggplot(data=penguins, aes(x=species, y=body\_mass\_g)) + geom\_bar(stat = "summary", fun= "mean", fill = "#0c4c8a")



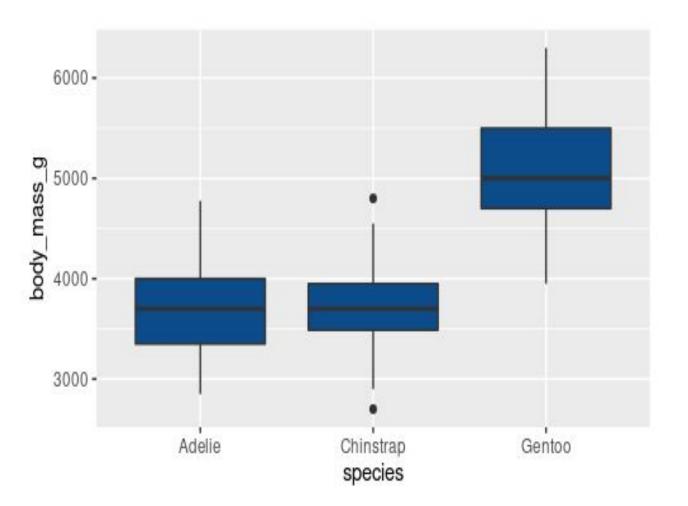
geom\_bar(stat= "summary",fun = "mean") we specified that we use a summary statistic, in particular the mean

## Histogram, barcharts, boxplots: boxplots



Can we have more information about the distribution of mass among species?

> ggplot(data = penguins, mapping = aes(x = species, y = body\_mass\_g)) + geom\_boxplot()



- thick line: median
- lower line: 25th percentile
- upper line: **75th percentile**
- whiskers: further non outlier points
- points: outliers

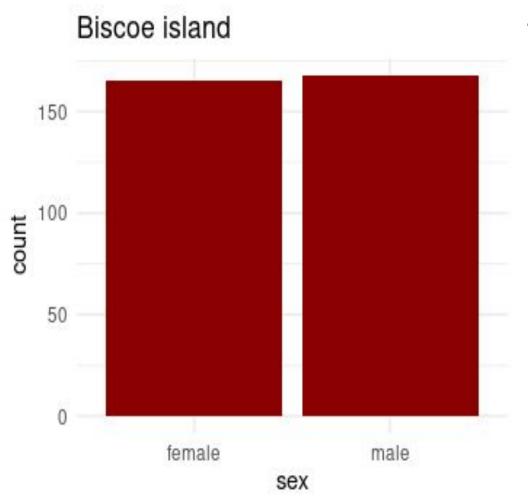


# 4 Statistical tests for proportions and means

## Statistical tests: one sample z-test for proportion



Is the female to male ratio for penguins statistically similar to the expected one (0.5)?



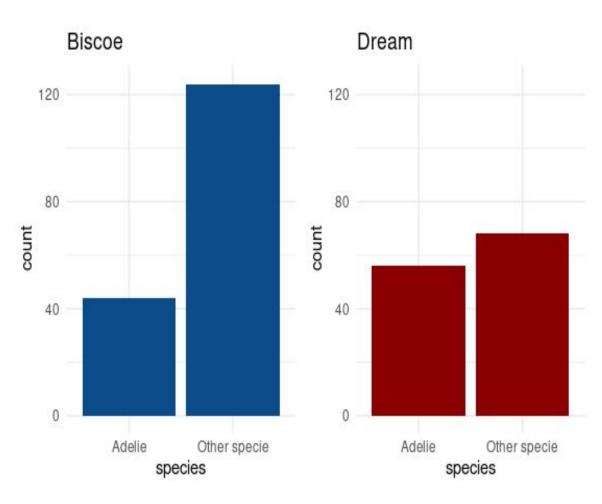
<u>prop.test</u> tests if an observed proportion is equal to a certain expected value (z-test).

```
test sex <- prop.test(
  x = 165, # number of successes (female)
  n = 333, # total number of trials (total num penguins)
  p = 0.5, # we test for prob = 0.5
  conf.level = 0.95 # confidence level
          1-sample proportions test with continuity correction
  data: 165 out of 333, null probability 0.5
  X-squared = 0.012012, df = 1, p-value = 0.9127
  alternative hypothesis: true p is not equal to 0.5
  95 percent confidence interval:
   0.4406707 0.5504259
p-value = 0.91 and the estimated proportion is [0.44,0.55] \rightarrow
              fail to reject H0 of 50% female
```

## Statistical tests: two sample z-test for proportion



Are proportions between Adelie and other species statistically similar on different islands?



<u>prop.test</u> also tests if proportions are similar between two groups (z-test 2 sided)

#testing for equality of species on Biscoe vs Dream
adelie <- c(44, 56)
total\_penguins <- c(168,124)</pre>

#p value << 0.05 we can reject H0 with high confidence
prop.test(adelie, total\_penguins)</pre>

2-sample test for equality of proportions with continuity correction

data: adelie out of total\_penguins
X-squared = 10.575, df = 1, p-value = 0.001146
alternative hypothesis: two.sided
95 percent confidence interval:
 -0.30668273 -0.07273355
sample estimates:
 prop 1 prop 2
0.2619048 0.4516129

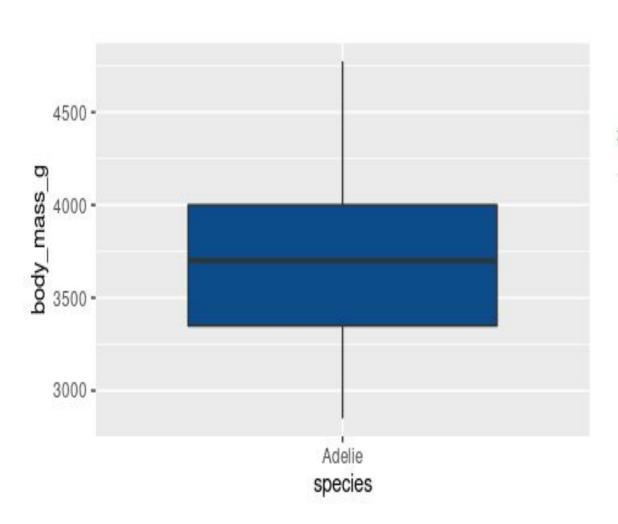
p-value =  $0.0011 \rightarrow$ 

We can reject the null hypothesis (proportions are statistically different)

#### Statistical tests: one sample t-test for means



Is the average mass of Adelie equal to 3.6 kg?



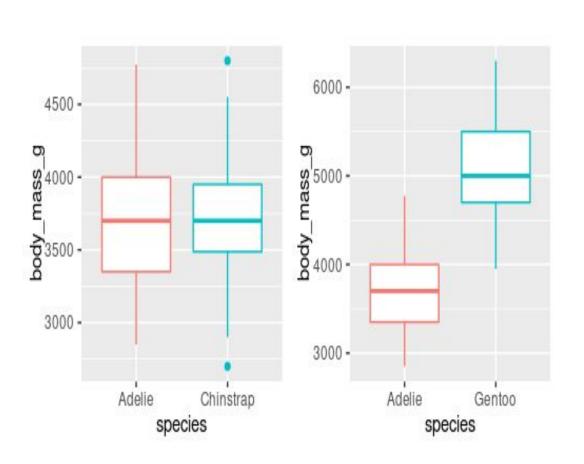
t.test performs one and two sample t-tests on vectors of data

```
#One Sample t-test with almost correct mean, unknown variance
test_right <- t.test(dat$body_mass_g,</pre>
                 mu = 3600,
                 alternative = 'greater')
             One Sample t-test
     data: dat$body mass g
     t = 2.6974, df = 150, p-value = 0.003894
     alternative hypothesis: true mean is greater than 3600
     95 percent confidence interval:
                     Inf
      3638.899
                        p-value = 0.004 \rightarrow
                 We can reject the null hypothesis
                (the mean is not stat. equal to 3.6kg)
                                                           21
```

## Statistical tests: two sample t-test for means



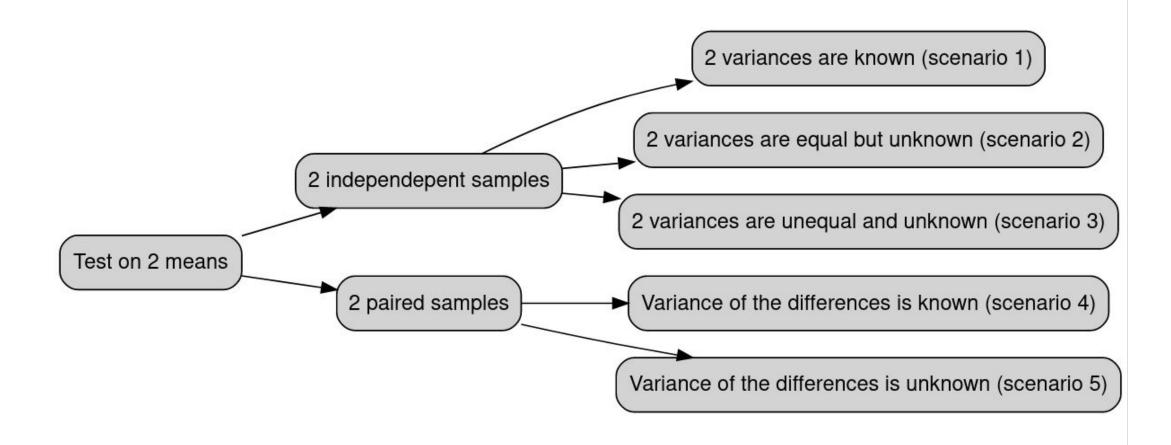
Is the average mass of Adelie penguins statistically different from those of Gentoo and Chinstrap?



t.test performs one and two sample t-tests on vectors of data

## Statistical tests: which t-test should I perform?





Source: "Stats and R" blog by Antoine Soetewey for this plot and a more general version of it



# 5 ANOVA

## ANOVA: motivation and quick reminder



The main aim of ANOVA is to compare more than 2 groups in a statistically sound way

Probability of observing one significative results due to chance for 3 groups

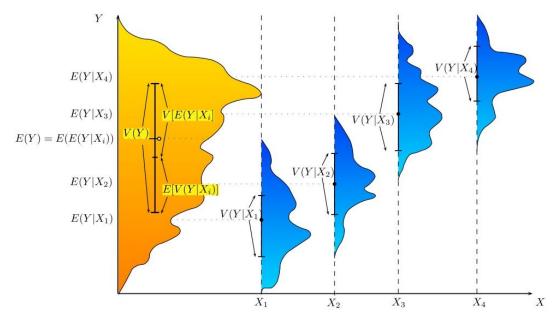
$$P(\text{at least 1 sig. result}) = 1 - P(\text{no sig. results})$$
  
=  $1 - (1 - 0.05)^3$   
=  $0.142625$ 

#### **ANOVA:** analysis of variance

 $\frac{variance_{between}}{variance_{within}}$ 

is larger than a certain threshold (5%) groups are considered different

- <u>Independence</u> of observations
- Normality for the distributions of the <u>residuals</u>
- Equality (or "homogeneity") of variances



## **ANOVA: visual analysis**



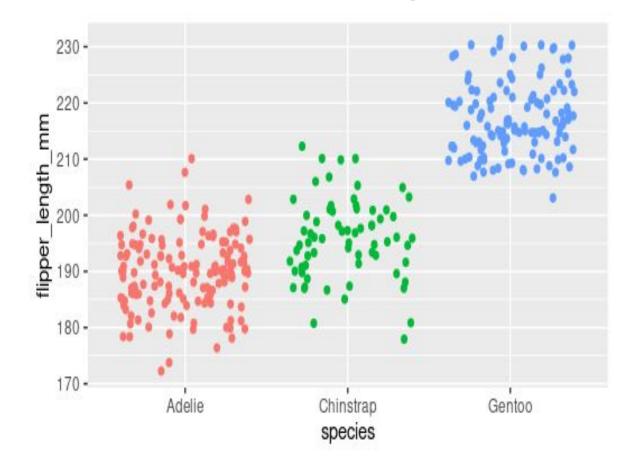
Do the 3 species have statistically significant different flipper lengths?

# #visualizing the flipper length per specie ggplot(dat) + aes(x = species, y = flipper\_length\_mm, color = species) + geom\_jitter() + theme(legend.position = "none")

geom\_jitter is a shortcut for geom\_point(position = "jitter").

It adds a **small amount of random variation** to the location of each point for visualization

#### **Gentoo seem to have longer flippers!**



## **ANOVA:** perform ANOVA with R



Do the 3 species have statistically significant different flipper lengths?

#### Test for Equal Means in a One-Way Layout

#### Fit an Analysis of Variance Model

```
# 1st method for ANOVA
                                                            # 2nd method for ANOVA (more info)
oneway.test(flipper_length_mm ~ species,
                                                            res_aov <- aov(flipper_length_mm ~ species,
           data = dat,
                                                                           data = dat
           var.equal = TRUE # assuming equal variances
                      > #ANOVA summary for this method. In this particular case
                      > # groups are sign. different since p is very small
                      > summary(res aov)
                                   Df Sum Sq Mean Sq F value Pr(>F)
                                    2 52473
                      species
                                               26237
                                                        594.8 <2e-16 ***
                      Residuals
                                 339 14953
                                                   44
                      Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
                      2 observations deleted due to missingness
```

We reject the H0 that the 3 means are equal due to the very low Pr(>F) (i.e. p)



## 6 Conclusions

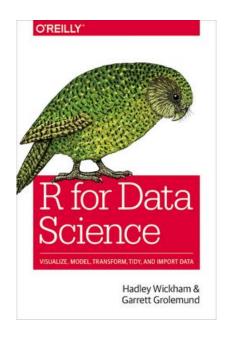
#### **Conclusions**

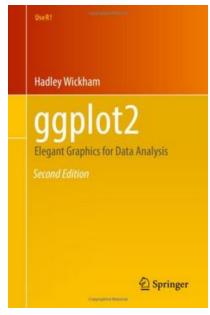


- R is a powerful language for statistical computing, easy to learn and with a vibrant community (R blog)
- some of the basics of R: installation, data types, operators, tibbles, ...
- ggplot for visualization is powerful but can get complex fast
- statistical tests are easy to perform in R, but be careful which one you choose!
- Please check important topics that we don't have the time to cover in this course (e.g. functions, loops, recycling rules, modelling, ...)

#### **Additional material**

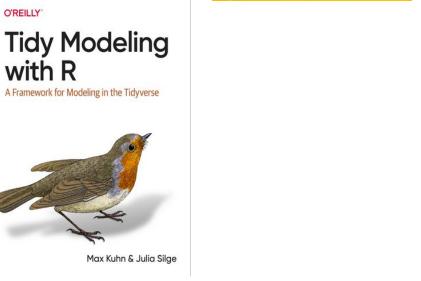












#### Contact me!





Github containing slides and code for this lecture



#### Do not hesitate to contact me

if you have any further question about this lecture

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My LinkedIn profile







# Thank you

